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Biomedical application of surface plasmon resonance biosensors (review)

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Over the last decade, surface plasmon resonance (SPR) becomes one of the major methods for the detection and investigation of affinity-based interactions in biochemistry, bioanalytical chemistry and biomedicine. The tremendous development of SPR use in biomedical applications during the last years is the reason why attention in this review is focused on the application of SPR for biomedical purposes. Biomedical applications take advantage of the exquisite sensitivity of SPR for the determination of DNA hybridization, diagnosis of virus-induced diseases, enzyme–substrate interactions, polyclonal antibody characterization, epitope mapping, protein conformation studies and label-free immunoassays. This review covers also some aspects of the evolution of surface plasmon resonance biosensors. The general principles of SPR sensor action are presented and major SPR formats are described. SPR biosensor employment for detection of various analytes ranging from small organics up to large protein complexes is reviewed. Immobilization methods used in the design of SPR biosensors are briefly described. Application of SPR for analysis of protein kinases as a major component in signal generation and transduction in eukaryotic cell is discussed. New ways of SPR application and new designs of hybrid SPR sensors are predicted and discussed. It is concluded that improvement of detection limits, multichannel performance, development of advanced recognition elements are the major tasks in developing new SPR sensors.

Key words: SPR, biosensor, immunosensor, immobilization, antigen–antibody reaction

INTRODUCTION

Rapid, comprehensive and accurate diagnostics is one of the most important topics of modern medicine, because it has a tremendous influence on the successful treatment of the patient. Today almost the entire biomedical analysis is performed employing bioassays and/or biosensors.

Biosensors are most promising in biomedical analysis since they can be easily integrated within microprocessor-based electronics (1). They allow an easy computation of signals and in particular cases even the diagnosis of some diseases and/or functional disorders. According to biochemical reactions exploited for analyte detection, biosensors might be divided into catalytic biosensors and affinity sensors. As the number of analytes detectable by affinity sensor is by several orders of magnitude higher, at present affinity sensors are more important for medical diag-

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nostics. The major classes of affinity sensors are: immunosensors (2), DNA sensors (3) and molecularly imprinted polymer-based sensors (4). The most promising are the affinity sensors that allow direct detection of analyte binding in real time. However, the transduction of analytical signal in this case is a challenging factor and just very few physical methods are really applied for direct measurements of analytical signal in real time. These are impedance spectroscopy (3), pulsed amperometric detection (4), quartz crystal microbalances (5), surface plasmon resonance (SPR) (6) and reflectometric interference spectroscopy (7).

Very promising in this case are SPR biosensors, since they are the most sensitive if compared to other transduction principle-based biosensors. Moreover, SPR biosensors can be applied for kinetic measurements of analytical signal, allowing a separate determination of the association and dissociation rate constants and thus a more accurate characterization of the kinetic reaction of an analyte in the sample of interest.

Over the recent years surface plasmon resonance has developed into a very useful technology with numerous applications. Current technical achievements in SPR lead to compete against application of immunoassays, which are commonly and widely used for determination of numerous important substances and offer low-cost tests of high specificity and sensitivity.

Since SPR biosensors are rapidly forcing into the biomedical analysis, the aim of this paper is to overview some SPR applications for biomedical and medical diagnostic purposes and to highlight the most promising directions for SPR-based biosensing.

BRIEF HISTORY OF SURFACE PLASMON RESONANCE

The phenomenon of anomalous diffraction on diffraction gratings due to the excitation of surface plasma waves was first described by Wood (8) in the beginning of the twentieth century. In the late sixties, optical excitation of surface plasmons by the method of attenuated total reflection was demonstrated by Kretschmann (9) and Otto (10). Since then, the application of surface plasmon resonance has been intensively studied and major properties assessed.

In the last two decades we have witnessed remarkable research and development activity aimed at optical sensors for the measurement of chemical and biological quantities. The first optical chemical sensors were based on the measurement of changes in absorption spectrum and were developed for the measurement of CO_2 and O_2 concentration (11). In these sensors a desired quantity is determined by measuring the refractive index, absorbance and fluorescence properties of analyte molecules or a chemo-optical transducing medium (12). The potential of surface plasmon resonance (SPR) for characterization of thin films (13) and monitoring processes at metal interfaces (14) was recognized in the late seventies. In 1982, the use of SPR for gas detection and biosensing was demonstrated by Nylander and Liedberg (15, 16). Liedberg et al. adsorbed an immunoglobulin G (IgG) antibody layer on a gold sensing film, resulting in the subsequent selective binding and detection of IgG (17). Most importantly, SPR has an inherent advantage over the other types of biosensors in its versatility and capability of monitoring binding interactions without the need for fluorescence or radioisotope labeling of the biomolecules. This approach has also shown promise in the real-time determination of concentration, kinetic constants, and binding specificity of individual biomolecular interaction steps. Antibody–antigen interactions, peptide/protein–protein interactions, DNA hybridization conditions, biocompatibility studies of polymers, biomolecule–cell receptor interactions, and DNA/receptor–ligand interactions can all be analyzed (18).

PRINCIPLES OF SURFACE PLASMON RESONANCE

Surface plasmon resonance is a charge-density oscillation that may exist at the interface of two media with dielectric constants of different signs, for instance, a metal and a dielectric. The charge density wave is associated with an electromagnetic wave, the field vectors of which reach their maxima at the interface and decay evanescently into both media. This surface plasma wave (SPW) is a TM-polarized wave (magnetic vector is perpendicular to the direction of propagation of the SPW and parallel to the plane of interface). The propagation constant of the surface plasma wave propagating at the interface between a semi-infinite dielectric and a metal is given by the following expression:

$$\beta = k\sqrt{\frac{\varepsilon_m n_s^2}{\varepsilon_m + n_s^2}}, \qquad (1)$$

where k denotes the free space wave number, ε_m is the dielectric constant of the metal ($\varepsilon_m = \varepsilon_{mr} + i\varepsilon_{mi}$), and n_s is the refractive index of the dielectric.

As may be concluded from Eq. (1), the SPW may be supported by the structure providing that $\varepsilon_m > n_s^2$ at optical wavelengths. This condition is fulfilled by several metals of which gold and silver are the most commonly used (19). Owing to high loss in the metal, the SPW propagates with high attenuation in the visible and near-infrared spectral regions. The electromagnetic field of the SPW is distributed in a highly asymmetric fashion, and the bulk of the

field is concentrated in the dielectric. The SPW propagating along the surface of silver is less attenuated and exhibits a higher localization of the electromagnetic field in the dielectric than SPWs supported by gold.

Generally, SPR optical sensor comprises an optical system, a transducing medium which interrelates the optical and (bio)chemical domains, and an electronic system supporting the optoelectronic components of the sensor and allowing data processing as well as a proper sample delivery system. The transducing medium transforms changes in the quantity of interest into changes in the refractive index, which may be determined by optically interrogating the SPR. The optical part of the SPR sensor contains a source of optical radiation and an optical structure in which an SPW is excited and interrogated. In the process of interrogating the SPR, an electronic signal is generated and processed by the electronic system. The major properties of SPR sensor are determined by the properties of the sensor's subsystems. The sensor sensitivity, stability, and resolution depend upon the properties of both the optical system and the transducing medium. The selectivity and response time of the sensor are primarily determined by the properties of the transducing medium (20).

As follows from Eq. (1), the propagation constant of SPW is always higher than that of optical wave propagation in the dielectric, and thus the SPW cannot be excited directly by an incidental optical wave at a planar metal–dielectric interface. Therefore, the momentum of the incidental optical wave has to be enhanced to match that of the SPW. This momentum change is commonly achieved using attenuated total reflection (ATR) in prism couplers (Figure).

As the excitation of SPW by optical wave results in resonant transfer of energy into the SPW, SPR manifests itself by resonant absorption of the energy of the optical wave. Owing to the strong concentration of the electromagnetic field in the dielectric (an order of magnitude higher than that in typical evanescent field sensors using dielectric wave guides) the propagation constant of the SPW, and consequently the SPR condition, is very sensitive to variations in the optical properties of the dielectric adjacent to the metal layer supporting SPW (transducing medium). Therefore, variations in the optical parameters of the transducing medium can be detected by monitoring the interaction between the SPW and the optical wave. The following main detection approaches have been commonly used in SPR sensors:

1. Measurement of the intensity of the optical wave near the resonance (21).

2. Measurement of the resonant momentum of the optical wave including the angular (22) and wavelength interrogation of SPR (23).

APPLICATION OF SPR FOR BIOMEDICAL PURPOSES

There is a need for detection and analysis of chemical and biochemical substances in many important areas including medicine, environmental monitoring, biotechnology, drug and food monitoring, military and civilian airborne biological and chemical agent testing, and real-time chemical and biological production process monitoring. Surface plasmon resonance sensor technology holds potential for applications in these areas.

The surface plasmon resonance phenomenon has been known for a long time. However, its application in biosensing is relatively new. The use of SPR for biosensing purposes was first demonstrated in 1983 by Liedberg et al. (17).

Biomedical applications take advantage of the exquisite sensitivity of SPR to the refractive index of the medium next to the metal surface, which makes it possible to measure accurately the adsorption of molecules on the metal surface and their eventual

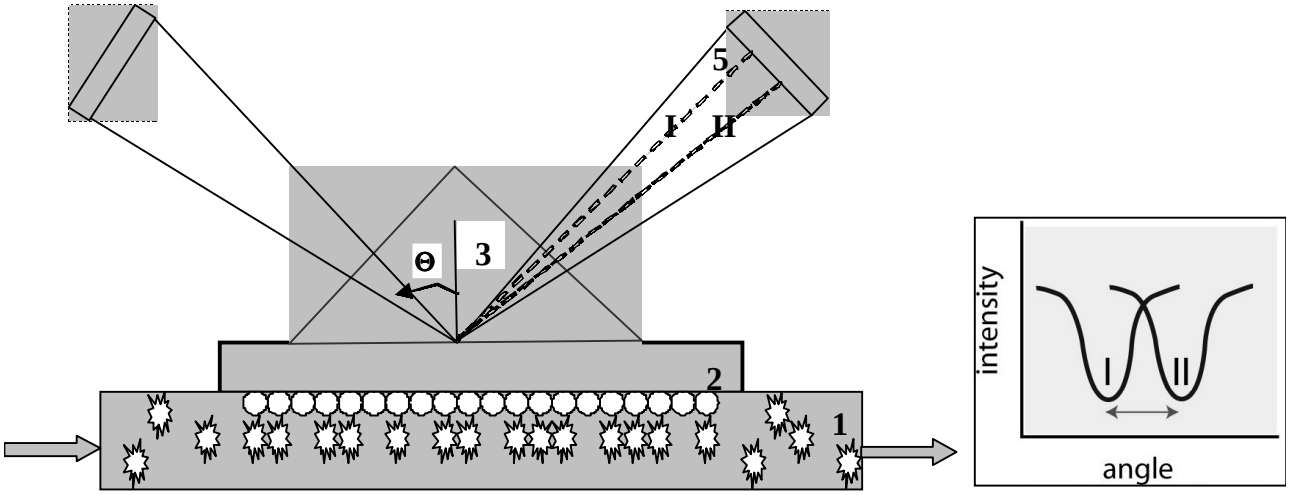

Figure. Most widely used configuration of SPR sensors. prism coupler-based SPR system: 1 – flow cell, 2 – the sensor surface is gold with attached ligand, 3 – prism, 4 – light source, 5 – light detector

interactions with specific ligands (24). During the last years a tremendous development of SPR use in biomedical applications emerged. Whilst several biosensor concepts have been developed, affinity biosensors using SPR have the merit to be the first sensor instruments and systems to be commercialized and hence have been made available to thousands of laboratories.

The most common application of biosensing SPR instruments is the determination of affinity parameters for biomolecular interactions (25). Chemically similar molecules can be detected by their biospecificity for an immobilized molecule. There is a linear relationship between the amount of bound material and the shift of the SPR angle (26). Any pair of molecules, which exhibit specific binding, can be adapted to SPR measurement. These may be an antigen and antibody, a DNA probe and complementary DNA strand, an enzyme and its substrate, oil and a gas or liquid which is soluble in the oil, or a chelating agent and metal ion.

The technique is applied not only to the real-time measurement of the kinetics of ligand–receptor interactions and to the screening of lead compounds in the pharmaceutical industry, but also to the measurement of DNA hybridization, enzyme–substrate interactions, in polyclonal antibody characterization, epitope mapping, protein conformation studies and label-free immunoassays. Conventional SPR is applied in specialized biosensing instruments. These instruments use expensive sensor chips of limited reuse capacity and require complex chemistry for ligand or protein immobilization.

Earlier works using SPR were focusing mainly on antigen–antibody interactions (27), the streptavidin–biotin reaction, and some IgG examinations (20). One of the new areas is the examination of protein–protein or protein–DNA interactions (28), even detecting conformational changes in an immobilized protein (29). A domain within the tumor suppressor protein APC has been examined regarding its biochemical properties (30), as well as the binding kinetics of human glycoprotein with monoclonal antibodies (31). Work has been done on the activator target in the RNA polymerase II holoenzyme (32). In addition to the examination of the structure–function relationship of antibacterial synthetic peptides (33), the binding conditions of the neuropeptide substance P to monoclonal antibodies have been examined, and equilibrium and kinetic studies reported (34). Even libraries are now being tested in order to determine binding affinities of a T-4 monoclonal antibody Fab fragment for thyroxine analogs (35). Epitope studies have been made in the case of characterization of recombinant hepatitis B surfaces with antigens (36). Another important area is membrane examinations as in the case of plasma membrane Ca^{2+} ATPase being a pump important for intracellular Ca^{2+} homeostasis (37). Another upcoming field is measurements to quantify T cell receptors in interaction with syngeneic or allogeneic ligands (38). Phage peptide libraries constitute a powerful tool for mapping the epitopes where antibody–peptide interactions are monitored by SPR (39).

SPR has been used to monitor such events as DNA hybridization (40), nuclear receptor–DNA interaction (41), immunoreactivity of antibody conjugates (42), peptide–antibody interactions (43), enzymatic turnover (44), detection of polymerase chain reaction products (45), characterization of proteins by epitope mapping with monoclonal antibodies (46), quantitative immunoassays (47), drug absorption extrapolation, drug–protein interactions (48), analysis of structure–function relationship of proteins and ligands (49), quantitative structure–activity relationship (QSAR) (50). SPR can be used to study tissue factor induced coagulation of whole blood and plasma, to study the conformation of immobilized proteins in various environments (51, 29). SPR sensor may be used for detection of hormones, drugs, steroids, immunoglobulins, viruses, whole bacteria, bacterial antigens, enzymatic, chemical, and gas adsorption as well as for the binding of metal ions to serum albumins (52, 53, 54).

The SPR sensor technology has been commercialized by several companies and has become a leading technology in the field of direct real-time observation of biomolecular interactions (52).

CHARACTERIZATION OF PROTEIN KINASE ACTION BY SPR

The progression of membranes and proteins through the stages and compartments of the secretory and endocytic pathways is a highly organized and regulated process. The maintenance of the overall architecture of endomembranes and the plasma membrane requires a balance of lipid flow into and out of various compartments, and proteins destined for diverse organelles or plasma membrane domains must be appropriately sorted and targeted, whereas resident proteins of specific pathway stages must be retained or retrieved. These events require the interplay of lipids, membrane proteins, soluble cytosolic and lumenal proteins, and cytoskeletal and motor proteins. Their internal coordination and external regulation are known to involve protein phosphorylation and small and heterotrimeric G-proteins (55).

cAMP-dependent protein kinases (PKAs) play a key role in many signal transduction processes, mediating the majority of the known effects of cAMP in the eukaryotic cell (87). PKA-dependent phosphorylation of nuclear and cytoplasmic substrates controls multiple cell functions, including motility, metabolism, differentiation, synaptic transmission, ion channel activities, growth, and coordinate gene

transcription (56, 57). Eukaryotic cells express multiple forms of PKA regulatory and catalytic subunits, which assemble together as different holoenzyme isoforms. Four different regulatory subunits (RIa, RIb, RIIa and RIIb) of PKA have been identified and serve to regulate catalytic activity by binding and inactivating the C-subunit. The C-subunit is released and activated upon binding four molecules of cAMP to the R-subunit dimer (56, 58). The characteristics of the PKA holoenzymes are largely determined by the structure and properties of their R subunits. C-PKAs show common kinetic features and substrate specificity (56). The R subunits are differentially distributed in mammalian tissues. RIa and RIIa are ubiquitous, whereas RIIb is expressed predominantly in endocrine, brain, fat and reproductive tissues (57). Type I PKA (containing RIa or RIb) is known to be mainly soluble; it has also been demonstrated to localize in proximity to membrane receptors such as antigen receptors on lymphoid cells and nicotinic acetylcholine receptors in neuromuscular junctions (59). In contrast, type II PKA (containing RIIa or RIIb) is primarily particular and associated with cytoskeletal elements and a number of organelles. In addition to the distinctive expression and distribution of the R subunits, they differ in their regulation and biochemical properties. The binding affinity to cAMP of RIIb *in vivo* is lower relative to RIIa and much lower compared to RIa (56). These data imply that holoenzymes containing RI or RII subunits (PKAI and PKAII) decode cAMP signals that differ in duration and intensity: PKAI is activated transiently by weak cAMP signals whereas PKAII responds to a high and persistent cAMP stimulation. Neurons and endocrine cells, which express predominantly PKAII, are adapted to persistent high concentrations of cAMP (57). A positive autoregulatory loop links the synthesis and accumulation of RIIb and the activation of nuclear cAMP signaling. Prolonged cAMP stimulation enhances RIIb expression and maintains PKAII content high in these cell types. The composition and specific biochemical properties of PKA isoenzymes account, in part, for differential cellular responses to discrete extracellular signals that activate adenylate cyclase (57).

Selectivity in PKA action may be mediated by particular pools of kinase compartmentalized at different subcellular loci through interaction with A-kinase anchoring proteins (AKAPs, reviewed in (60), which target PKA towards specific substrates. Targeting of PKA to various subcellular loci is mediated by interaction of the R-subunits with different AKAPs (60). While the subcellular targeting of RI via mono- or dual-specific AKAPs is emerging (61), it is well known that RII can be specifically bound to AKAPs that bind exclusively RII or both RII and RI. AKAPs have been found to be associated, *e.g.*, with the plasma membrane, the endoplasmic reticulum, the nuclear membrane, microfilaments, microtubules, mitochondria, peroxisomes, centrosomes, or postsynaptic sites and are implicated in the PKA regulation of physiological events such as sperm motility, insulin secretion, the modulation of neurotransmitter receptors and ion channels, and the exocytosis of water channel-carrying vesicles in kidney cells (62).

The biochemical basis for the distinct distribution of PKA isozymes is based on the specificity and affinity in PKA interaction with available AKAPs. It is important to develop a molecular understanding of the subcellular distribution and specific functions of PKA isozymes. Surface plasmon resonance is a convenient method for macromolecular interactions. Herberg et al. developed methods to assess the apparent binding constants of the R–AKAP interaction (63). They immobilized the R-subunit via the cAMP-binding sites on a modified cAMP surface, and examined the association and dissociation of AKAP proteins with all R-subunit isoforms by surface plasmon resonance. They examined the PKA-interaction of AKAP79, have reported to bind both RIIa and RIIb, with the four R-subunits (RIa, RIb, RIIa and RIIb) and compared it with that of AKAP95, have reported to bind more selectively RIIa, and with that of S-AKAP84/D-AKAP1 (AKAP121, AKAP149), which was reported as a dual-specific AKAP binding both RI and RII and showed selectivity in AKAP binding for the different R-subunits. Apparent rate- and equilibria binding constants and EC_{50} values for competitor peptides were determined (63). The mechanism of redistribution of RIIa and the functional implications of the detachment of RII RIIa from centrosomes at mitosis were studied (64). Cell lines stably expressing wild type and mutated RIIa(T54E) on a RIIa-deficient background were analyzed. Mutated RIIa(T54E) was not phosphorylated by CDK1 and was retained at the mitotic centrosomes of the transfectants. CDK1 phosphorylation of wild-type RIIa lowered the affinity for AKAP450 *in vitro* and dissociated RII RIIa from purified centrosomes. This suggests that CDK1 phosphorylation serves as a molecular switch that regulates RIIa association with centrosomal AKAPs. The identification and characterization of a new A-kinase anchoring protein 18 isoform (AKAP18, AKAP18d) were reported, and evidence for its involvement in the vasopressin-induced aquaporin-2 (AQP2) shuttle in renal principal cells was provided (65).

Using the method described above, neurobeachin, a novel neuron-specific protein, was characterized as an AKAP (66). Neurobeachin was identified as a component of synapses, but most of it proved to be associated with tubulovesicular endomembranes throughout neuronal cell bodies and dendrites and concentrated near the *trans*-Golgi. It is a large mul-

tidomain protein which is recruited from cytosol to Golgi-near membranes in a coat protein-like, GTP-dependent, and brefeldin A (BFA)-sensitive fashion, suggesting an involvement in membrane traffic. Neurobeachin also contains a BEACH-WD40 sequence module. This makes it the third member to be characterized of an emerging family of ~10 distinct mammalian proteins with BEACH-WD40 domains. The prototype of this family is lysosomal trafficking regulator (LYST), a cytosolic protein important for lysosomal biogenesis and implicated in protein sorting between endosomes, lysosomes, and the plasma membrane (66).

Surface plasmon resonance was used to characterize a novel human protein kinase, PrKX, which is related to the catalytic subunit of cAMP-dependent protein kinases but is distinct from isoforms Ca, Cb and Cg. PrKX interaction with known inhibitors of cAPK and its regulation by the second messenger cAMP *in vitro* and *in vivo* were assessed (67).

DETECTION OF LOW MOLECULAR WEIGHT ANALYTES BY SPR

Some biologically active low molecular weight materials have a high impact on the regulatory processes of organisms (68). It is the reason why detection of those analytes plays a significant role in biomedicine and bioanalytical chemistry (69). Here direct analyte detection methods are very requested.

Since the SPR measures the mass of material binding to the sensor surface, very small analytes (Mr < 1000) give very small responses. Recent improvements in the signal-to-noise ratio have made it possible to measure the binding of such small analytes. However, a very high surface concentration of active immobilized ligand (~1 mM) is needed, and this is difficult to achieve (for review, see ref. 70).

Furthermore, at such high ligand densities accurate kinetic analysis is not possible because of mass-transport limitations and rebinding. Thus, in the majority of the described cases only equilibrium analysis is possible with very small analytes, and only under optimal conditions (71).

Immobilization of low-molecular-weight antigen can be advantageous in terms of the subsequent instrument response obtained upon binding a macromolecular antibody. However, immobilization of low-molecular-weight antigens can be difficult. Low-molecular-weight antigens generally have a few functional groups available for coupling to the biosensor surface and may require chemical modification to be incorporated. Low-molecular-weight antigen immobilization is also difficult to follow directly, since the instrument response will be small due to the limited mass of the antigen (72).

In general, the detection of small molecules such as glucose (180 Da) by SPR can be difficult; low molecular weight compounds may have insufficient mass to effect a measurable change in the refractive index. For this reason, early SPR papers used indirect competition to monitor binding of ligands less than 5000 Da to immobilized receptors (73). However, Helen V. Hsieh et al. study describes the direct detection of glucose using engineered glucose/galactose-binding protein (GGBP) coupled to SPR biosensor (74). The direct binding assay yields both kinetic and affinity parameters and has been used to characterize small-molecule inhibitors binding to proteinase (75), which is an essential enzyme for virion development. Several groups have used the biosensor in a competition analysis mode to determine the abilities of various small molecules, peptides and proteins to inhibit the gp160–CD4 (the T-cell receptor) complex formation (76).

Recently, improvement in SPR instrumentation has enabled detection of small molecules, such as drugs (≥138 Da) binding to human serum albumin (48) and small oligosaccharides (<1000 Da) binding to an antibody (74).

CONCLUSIONS

Today, the commercially available biosensors are rapidly forcing into the area of biomedical monitoring market aiming primarily at research and medical diagnostics laboratories. In order to faster reach out from specialized laboratories and centralized testing sites and gain a fair share of the biomedical monitoring market, SPR sensors have to compete with existing technologies on the basis of factors such as low cost, ease of use, robustness, sensitivity, and stability. It is envisaged that this will drive research and development of SPR-sensing devices in the following directions: (i) improvement of detection limits; (ii) multichannel performance; (iii) development of advanced recognition elements, since some biomedical samples are semi-transparent for light; the combination of SPR with electrochemical methods may a bring more reliable information on analyte concentration and other properties. Application of SPR hybridized with electrochemical detection might be especially useful for investigations of blood serum and other colored biological samples.

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PAVIRÐIAUS PLAZMONØ REZONANSO BIOSENSORIØ TAIKYMAS BIOMEDICINOJE

Santrauka

Pastaràjá deðimtmetá pavirðiaus plazmonø rezonansas (PPR) tapo vienu svarbiausiø metodø tiriant ir nustatant afinines sàveikas biochemijoje, bioanalizëje bei biomedicinoje. Pagrindinis PPR biosensoriø privalumas – didelis jautrumas registruojant DNR hibridizacijà, diagnozuojant virusines ligas, tiriant fermento-substrato sàveikas, ávertinant polikloninius antikûnus, analizuojant baltymø konformacijà bei vykdant imunoanalizæ. Kadangi PPR biosensoriø naudojimas biomedicinoje pastaraisiais metais ypaè iðaugo, ðiame straipsnyje buvo apþvelgtos PPR biosensoriø pritaikymo galimybës bei kai kurios jø savybës, ávertintas PPR biosensoriø panaudojimas nustatant ávairias analites – tiek maþø organiniø molekuliø, tiek dideliø baltymø kompleksø. Apraðytas PPR biosensoriø veikimo principas bei imobilizavimo metodai, naudojami ðiø biosensoriø konstravimui. Iðsamiai aptartas PPR metodo panaudojimas proteinkinaziø, dalyvaujanèiø daugelyje signalo perdavimo procesø eukariotinëse làstelëse, tyrimams. Ávertinti naujausi PPR metodo taikymo bûdai, naujø hibridiniø PPR biosensoriø konstravimo galimybës. Aptarti pagrindiniai tolimesnio PPR biosensoriø tobulinimo uþdaviniai.